

REVIEW

Cellular Aspects of Oocyte Growth in Teleosts

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ABSTRACT—In teleosts, the transformation of oogonia into oocytes apparently occurs within the germinal regions of the luminal epithelium of the ovary. As observed to particular advantage in syngnathans, prefollicle cells surround each oocyte, which is arrested in meiotic prophase, and the entire oocyte-follicle cell complex buds off the germinal nest as a primordial follicle. Oocyte growth within the follicle is due, to some extent, to the accumulation of normal cytoplasmic components; however, the preponderant mechanisms that contribute to oocyte growth are the endogenous synthesis of cortical alveoli, the accumulation of hepatically derived yolk protein (vitellogenesis) and in some (particularly marine) teleosts, a pronounced water uptake, which occurs concomitant with the resumption of meiosis (oocyte maturation). Our current understanding of these events is discussed and a new perspective on oocyte staging is presented, which is based on data indicating that the cellular events of oocyte growth do not sequentially replace one another, but rather are initiated sequentially and remain active throughout oocyte development.

INTRODUCTION

The production of viable eggs is obviously needed for species survival. Interest in teleost reproduction, therefore, has increased in recent years as teleosts have become appreciated for a number of commercial reasons. Frequently it is desirable to know the reproductive condition of a population of fish and this is often monitored either by representative sampling of individual fish or, in large fish, by a relatively simple biopsy through the genital pore (e.g., [1]). In either case, a knowledge of oocyte development is a prerequisite for a proper evaluation of reproductive condition and several staging systems have been used for different teleosts [2-8]. We have previously given a detailed review of the events of teleost oocyte growth and development [9] and will here simply summarize the various sequential processes involved along with related considerations,

emphasizing some of the newer information that has recently emerged. This review paper is intended to supplement the several monographs on various aspects of oocyte growth that have been published in the last few years [10-22] and will not attempt to cover endocrinological aspects of oocyte growth and maturation (for which, see [23]). In summarizing the cellular events of oocyte growth, examples will be drawn from the prevailing literature on a variety of teleosts, with some bias toward a few species that we have investigated in our own laboratories.

OOGENESIS AND FOLLICULOGENESIS

Apparently, oogonia can be found throughout the life of the female in most teleosts. These mitotically dividing cells represent a stem-cell population that gives rise to oocytes and ultimately to eggs. Discrepancies exist in the literature concerning the location of oogonia within the ovary, the seasonality of oogonial proliferation and the

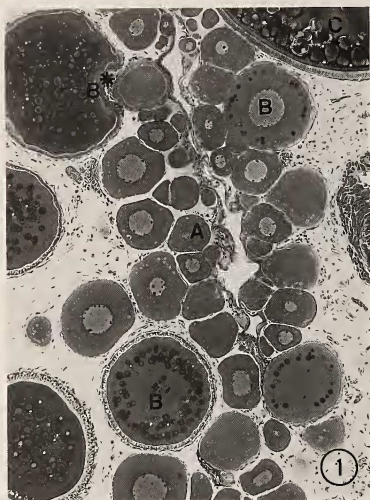


FIG. 1. Light micrograph of a section through an ovigerous fold of the ovary of the killifish, *Fundulus heteroclitus*, showing randomly arranged follicles in different stages of development. A, primary growth stage; B, B¹, B*, early, mid, and late cortical alveolus stages respectively; C, vitellogenic stage. Tissue was embedded in Histo-resin and the section was stained with toluidine blue. $\times 80$.

timing of oogenesis *per se* [24]. In mature ("ripe") ovaries of many fish, oocytes of variable size are randomly distributed (Fig. 1) and oogonia and early oocytes occur in nests embedded in the wall of ovigerous lamellae [8, 25–27]. Because of their small size, they are hard to locate and thus many conclusions in the literature regarding oogenesis may be compromised by periodic observational difficulties. A particularly useful adult material for studying oogonia and early oocyte stages is provided by syngnathans (pipefish and seahorse), where a sequential pattern of follicle development is observed. In the pipefish, *Syngnathus scovelli*, this pattern begins at the "germinal ridge", with a gradient of follicles of increasing developmental age extending to the mature edge (Fig. 2, [7, 28]).

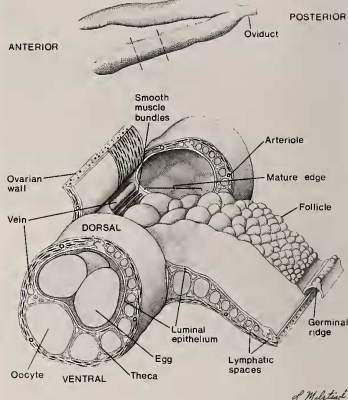
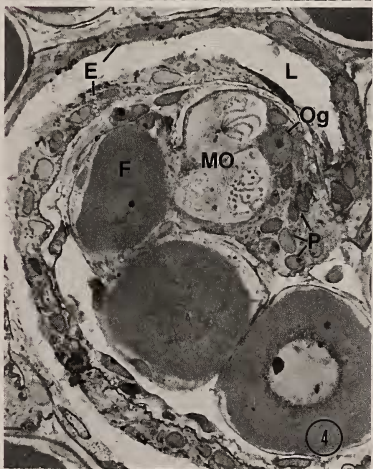


FIG. 2. Schematic summary of the ovary of the pipefish, *Syngnathus scovelli*. The upper diagram indicates the paired ovaries which join to give rise to the oviduct. The dashed line indicates a segment of the ovary that is enlarged in the lower diagram maintaining *in situ* relations. The middle section of the ovary has been unfurled to allow visualization of the follicles and ovarian lumen. Note that a strip of luminal epithelium has been removed except near the germinal ridge along with the underlying connective tissue elements to visualize the follicle progression in the ovarian sheet. The association of the luminal epithelium with the germinal ridge is indicated. The germinal ridge in the reflected segment of the luminal epithelium depicts early follicles in various stages of separation. The sandwichlike nature of the ovary is apparent in the unfurled region with follicles lying between the ovarian wall and the luminal epithelium. This diagram highlights features of the ovary and is not drawn fully to scale. From Begovac and Wallace [28].

In the seahorse, *Hippocampus erectus*, each of the ovaries has two germinal ridges, which run the length of the ovary on opposite sides of the ovarian sheet, and sequentially developing follicles arising from each grow towards a shared mature region (Fig. 3). In both cases, the germinal ridges contain



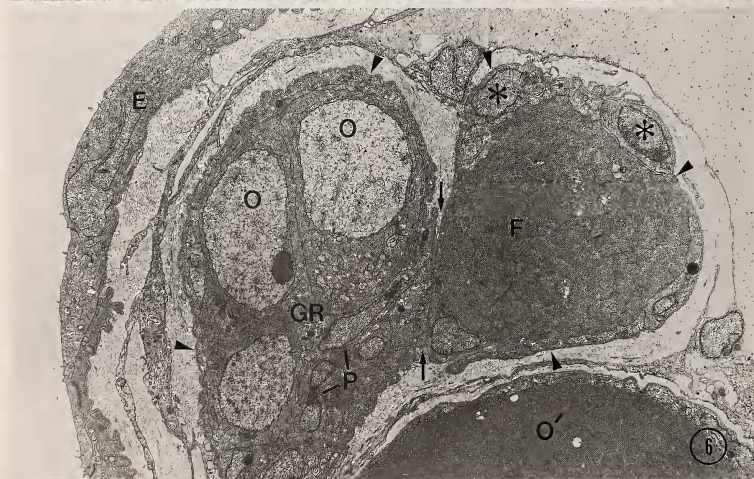
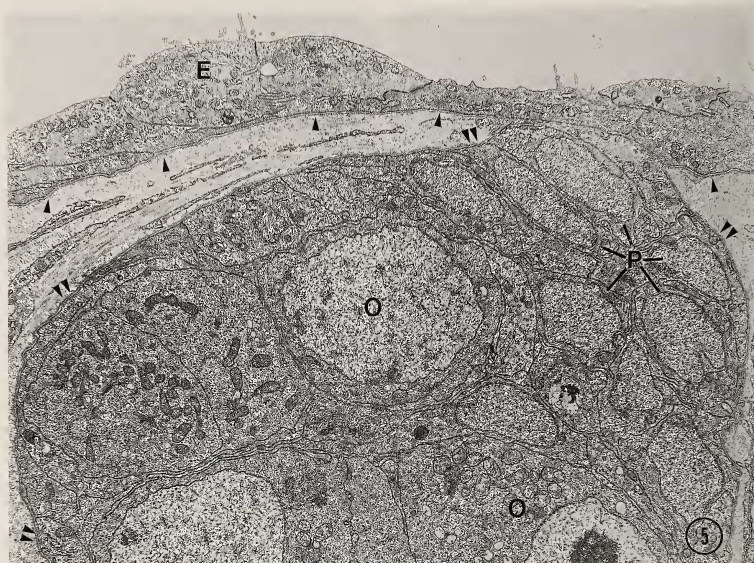
the germinal cells (oogonia and oocytes up to early diplotene) together with somatic prefollicle cells (Figs. 4–6) and, because of the exceptional accessibility of the germinal ridges, syngnathans are well suited for studies on the early events of oogenesis.

Until quite recently, the origin of germ cells and pre-follicle cells in teleosts has been speculative, being based primarily on light microscopic observations (e.g., [27, 29–31]). These studies suggested that germ cells were derived from the germinal (luminal or coelomic) epithelium or the underlying connective tissue cells. A more recent ultrastructural study of the killifish, *Fundulus heteroclitus*, suggested the latter [32]. Both mesenchymal and epithelial cells have also been purported to give rise to the prefollicle cells in a variety of fish. Recent ultrastructural studies on *S. scovelli*, however, have indicated that oogonia, the earliest oocytes, and the prefollicle cells are all derived from the luminal epithelium, i.e., they reside within an outpocketing of this ovarian compartment [28]. Figure 5 supports the notion of a single compartment and reveals that the basal lamina surrounding the germinal ridge is continuous with the basal lamina underlying the luminal epithelium.

The transformation of oogonia to oocytes is referred to as oogenesis. In syngnathans, oocytes are formed from oogonia within the germinal ridge as they enter the early stages of meiotic prophase (Fig. 4). During this period, DNA replication occurs (pre-leptotene), homologous chromosomes pair and begin to condense (leptotene, zygotene), these pairs subsequently shorten and thicken to

FIG. 3. Light micrograph of part of a transverse section through the ovary of the seahorse, *Hippocampus erectus*, showing two germinal ridges (arrowheads) and sequentially arranged growing follicles arising from each. O, oocytes; L, ovarian lumen. Tissue was embedded in Historesin and the section was stained with toluidine blue. $\times 95$.

FIG. 4. Light micrograph of a section through a germinal ridge region of an ovary from *H. erectus*. Within the germinal ridge prefollicle cells (P), an oogonium (Og) and meiotic oocytes (MO) with visible chromosomes are apparent. F, developing follicle that has already separated from the germinal ridge; L, ovarian lumen; E, luminal epithelium. Tissue was prepared as for Fig. 3. $\times 950$.



form synaptonemal complexes (pachytene), and finally the chromosomes take on a "lampbrush" configuration (diplotene), which is sometimes difficult to detect in routine histological preparations. Collectively, these events are referred to as the "chromatin nucleolus stage of primary oocyte growth". We have found that soon after the pipefish oocyte enters diplotene, it becomes enveloped by a continuous layer of follicle cells prior to leaving the germinal ridge (Fig. 6). Arrested in late diplotene of first meiotic prophase, the oocyte then begins an extensive period of growth concomitant with the differentiation of its follicular components and this is generally called the "perinucleolus stage of primary oocyte growth".

Soon after arrest in prophase, ribosomal genes are amplified and multiple nucleoli appear, although some alterations of this process have been described [33]. Although the size and morphology of the multiple nucleoli are variable amongst teleosts, their presence appears to be universal; they usually lie in the peripheral region of the enlarging oocyte nucleus, which is from this point on generally referred to as the "germinal vesicle" (Fig. 7). At this stage, the lampbrush configuration of the chromosomes becomes more apparent, indicating prominent transcription of heterogeneous RNA in addition to the ribosomal RNA provided by the multiple nucleoli [34]. The former is processed into poly(A)-containing RNA, the so-called "maternal message" (mRNA), as it moves from the germinal vesicle into the cytoplasm. Where quantified, the amount of maternal mRNA that is found in full-grown oocytes is already present by the end of primary oocyte growth [34, 35]. Aggregations of basophilic or electron dense material (nuage) is apparent in the perinuclear cytoplasm and has been shown to contain ribonucleoprotein [36, 37].

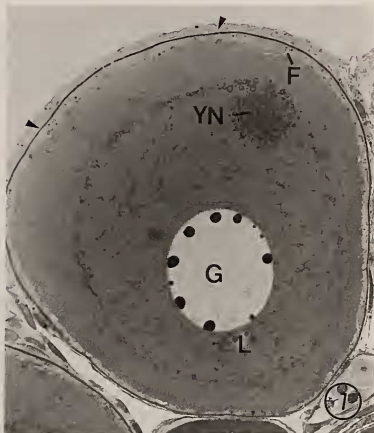
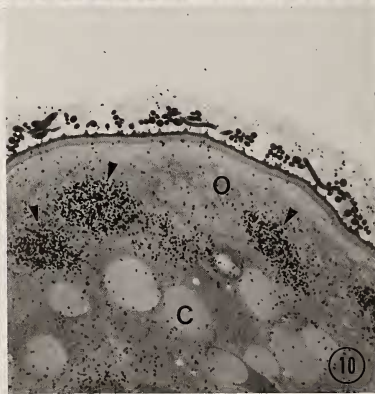
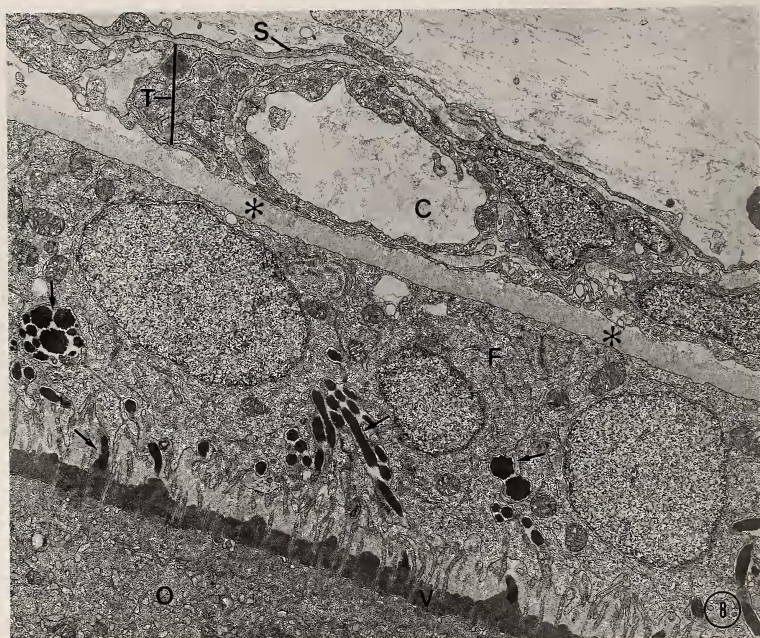


FIG. 7. Light micrograph of a section through a follicle in the perinucleolus stage of primary oocyte growth from the seahorse, *H. erectus*. The oocyte contains a distinct yolk nucleus (YN) and multiple nucleoli (G) are visible at the periphery of the germinal vesicle (G). A darkly stained basement membrane (arrowheads) separates the follicle cells (F) from the remaining follicular investments. L, lipid droplets. Tissue was embedded in Histo-resin and the section was stained with toluidine blue. $\times 565$.

A rather prominent feature of this stage is the formation of the "yolk nucleus" or "Balbani body" (Fig. 7), which was first described in teleosts by Hubbard [38]. At the ultrastructural level, this variably sized structure appears to be composed of aggregations of ribonucleoprotein particles associated with a heterogeneous population of cytoplasmic organelles, such as mitochondria, multivesicu-

FIG. 5. Electron micrograph of part of the germinal ridge from *S. scovelli*. Note that the basal lamina (single arrowheads) underlying the luminal epithelial cells (E) is continuous with the basal lamina (double arrowheads) that completely surrounds the germinal ridge. Groups of oocytes (O) and prefollicle cells (P) can be seen within the germinal ridge. $\times 5,700$. From Begovac and Wallace [28].

FIG. 6. Electron micrograph of an early follicle (F) separating from the germinal ridge (GR) in the ovary of *S. scovelli*. Definitive follicle cells (*) surround the oocyte as it "pinches" away (arrows) from the ridge. The basal lamina (arrowheads) can be followed and seen to enclose the entire germinal ridge and young follicle. Within the germinal ridge, prefollicle cells (P) and meiotic oocytes (O) are evident. E, luminal epithelium; O', an oocyte in a definitive follicle of the early follicle progression. $\times 18,500$. From Begovac and Wallace [28].



lar bodies, endoplasmic reticulum, and Golgi elements (see review by Guraya [39]). The Balbiani body initially forms in a juxtanuclear position and subsequently migrates to the oocyte periphery where it disperses into small fragments [40, 41]. These events have been followed in living oocytes by vitally staining with rhodamine 123 or acridine orange, which indicate mitochondria- and lysosome-related structures, respectively [7]. Although the existence of the Balbiani body is well documented, its functional significance is not fully understood, but most opinion suggests it is involved in the extensive fabrication of organelles that occurs during primary oocyte growth [39].

By the end of the primary growth stage a multilayered follicle has formed (Fig. 8). The follicle consists of a) an oocyte surrounded by a sheath of follicle (or granulosa) cells that rests on a prominent basal lamina, b) a vascularized thecal layer containing capillaries within a connective tissue meshwork, and c) a thin surface epithelium, in which the cells are connected by occluding junctions that prevent the diffusion of macromolecular material from the ovarian lumen into the follicle or vice versa. In many teleosts, formation of the vitelline envelope (variously referred to as the zona pellucida, zona radiata, chorion, or primary envelope) begins during the perinucleolus stage of oocyte growth and electron-dense material is observed at the ultrastructural level to be accumulating between short microvilli that project from the oocyte surface towards the overlying follicle cells (see Fig. 19a below).

During the primary growth stage in most teleosts, the oocyte grows from a diameter of approximately 10–20 μm at leptotene to a diameter ranging from 100–200 μm . The elaboration of normal cytoplasmic organelles and the accumulation of huge amounts (for a single cell) of cytoplasmic RNAs and proteins is responsible for approximately a thousand-fold increase in oocyte volume and a noticeable decrease in nucleo-cytoplasmic ratio. It is important to realize, however, that ovaries containing oocytes only in the primary growth stage are still relatively small, having gonadosomatic indices of <2 , and thus these ovaries are generally perceived as immature.

CORTICAL ALVEOLUS STAGE

The first distinct cytoplasmic structures that are uniquely associated with teleost oocytes at the light microscopic level are the cortical alveoli (Fig. 9), sometimes referred to as "yolk vesicles", "cortical vesicles", "intravesicular yolk", "endogenous yolk", and a variety of other terms [42, 43]. Initially, these spherical structures appear circumferentially at various depths in the cytoplasm (depending on species) and generally stain for both protein and carbohydrate [42]. As they enlarge to a size sometimes in excess of 50 μm in diameter, the cortical alveoli become more difficult to preserve during routine tissue preparation for microscopy, and in suboptimally fixed oocytes they often lose their staining properties and appear empty (vacuolar). By the end of this stage, cortical

Fig. 8. Low power electron micrograph of the follicle wall and underlying oocyte (O) from *F. heteroclitus*. The follicle is covered by a surface epithelium (S) made of squamous epithelial cells that are connected by tight junctions. The theca (T) consists of a network of capillaries (C) within a connective tissue stroma and is separated from the follicle cells (F) by a distinct basal lamina (*). Note the electron-dense fibrils (arrows) lying within spaces between adjacent follicle cells and attaching to the outer region of the developing vitelline envelope (V). $\times 8,830$.

Fig. 9. Light micrograph of a section through a cortical alveolus-stage follicle from *F. heteroclitus*. Multiple nucleoli are apparent within the germinal vesicle (G) and variable-size cortical alveoli (*) are randomly arranged within the oocyte. Lipid droplets (arrows) are readily distinguished from the cortical alveoli because the fixation solution contained osmium tetroxide. Tissue was embedded in JB-4 resin and the section was stained with toluidine blue. $\times 130$.

Fig. 10. Autoradiograph of a cortical alveolus-stage follicle that was incubated for 12 hr in fish saline containing 100 $\mu\text{Ci}/\text{ml}$ [^3H]glucose followed by a 12 hr incubation in fish saline containing an excess of unlabeled glucose. The endogenous synthesis of a cortical alveolus glycoconjugate is indicated by the high concentration of grains over cortical alveoli (C) that were formed prior to exposure to [^3H]glucose. Tissue was embedded in Epon, the slide was exposed for 7 days prior to development and was subsequently stained with toluidine blue. $\times 690$.

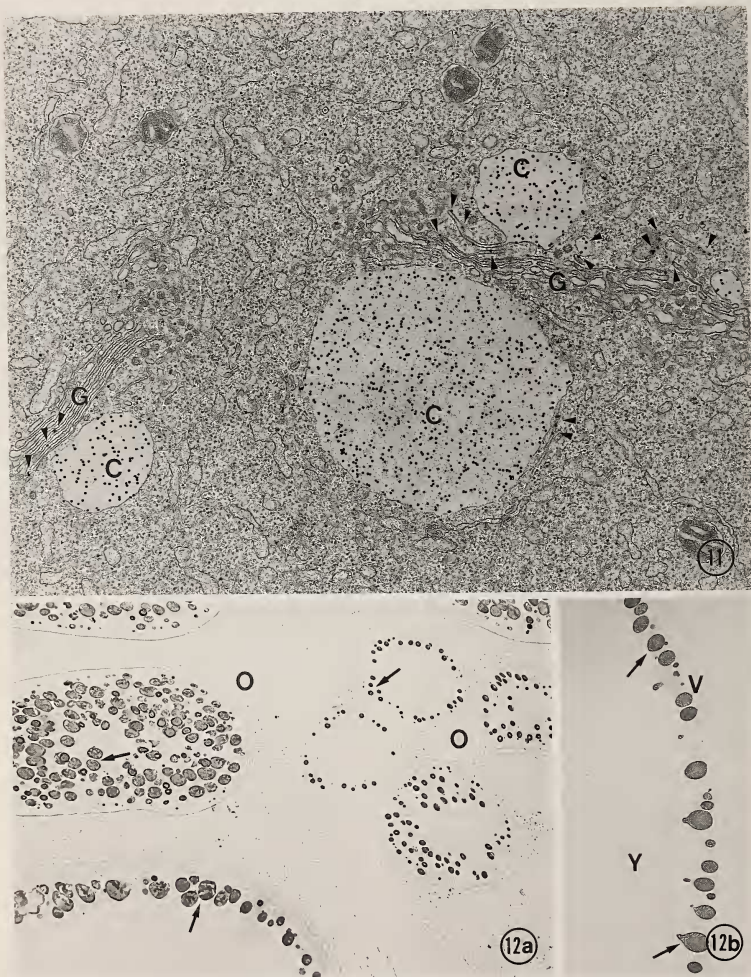


FIG. 11. Electron micrograph of a cortical alveolus-stage oocyte from *F. heteroclitus* that demonstrates immunoreactivity within Golgi complexes (G) and cortical alveoli (C) after treatment with an antibody raised against a component of cortical alveoli. The section was first treated with antiserum raised against a >200-kDa glycoconjugate that was derived from a cortical alveolus-stage oocyte and then secondarily treated with Protein

alveoli almost entirely fill the oocyte cytoplasm (Fig. 1), but in subsequent stages they continue to form and are displaced to the periphery of the oocyte by yolk protein, which accumulates centripetally [9, 42–44]. As a response to fertilization [45–48], cortical alveoli release their contents from the egg surface, and hence are homologous to “cortical granules” that are ubiquitous in eggs of other vertebrates and invertebrates [49]. Cortical alveoli, therefore, do not contain yolk (utilized by the embryo for nutrition) and should never be referred to in any way as yolk inclusions.

The formation of cortical alveoli has been examined in developing oocytes of a variety of teleosts and a common conclusion from these studies is that a carbohydrate-containing component of these vesicular structures is endogenously produced [43]. Recent studies have shown that cortical alveoli in several unrelated fish contain lectins with specific sugar-binding properties [50–52], the functions of which are unknown. Early reports had shown that oocytes of the zebrafish (*Brachydanio rerio*) synthesized a glycoprotein component of cortical alveoli [53, 54] and this has been followed more recently by a similar finding for the killifish, *F. heteroclitus* [42]. Autoradiographs (Fig. 10) made of follicles incubated in the presence of [^3H]glucose indicate that a carbohydrate component of cortical alveoli is endogenously produced within cortical alveolus-stage oocytes [42], and ultrastructural studies implicate the Golgi and/or the endoplasmic reticulum in this process [40, 42, 55–58]. This can be seen in Figure 11, where the Golgi complex and associated vesicles reveal immunocytochemical labeling with antibody directed against a >200-kDa glycoconjugate present within cortical alveoli [43]. Antibodies raised against purified ovarian lectins [52] and against cortical alveolus-derived glycoconjugates [43, 59] have been used to definitively establish the related-

ness of cortical alveoli present in eggs with what have generally been called “yolk vesicles” in early oocytes (Fig. 12). Taken together these studies indicate that yolk vesicles and cortical alveoli are identical in structure and composition and lead us to suggest that the term “yolk vesicle” not be used in future publications [43].

The most detailed analyses of cortical alveoli have been made by Inoue and colleagues [60, 61] for eggs of the rainbow trout, *Salmo gairdneri*. These investigators found the major cortical alveolus-associated component to be an unusual 200-kDa polysialoglycoprotein that has a core made of 25 tandem repeating tridecapeptide units with 0-linked glycan side chains composed of large blocks of sialic acid. They have also demonstrated that after fertilization and the concomitant exocytosis of cortical alveoli, the 200-kDa polysialoglycoprotein depolymerizes to 9-kDa subunits [62].

VITELLOGENESIS

Since yolk proteins contribute >80–90% of the dry weight of eggs in most lower vertebrates, including teleosts, vitellogenesis represents a major aspect of oocyte growth [17]. Many studies conducted primarily on amphibians and birds have indicated that the sequence of events that exclusively contributes to vitellogenesis involves 1) the hepatic synthesis and secretion of vitellogenin, the yolk protein precursor, in response to circulating estrogen, 2) the delivery of vitellogenin via the maternal circulation to the surface of the growing oocyte, 3) selective uptake of vitellogenin by receptor-mediated endocytosis, and 4) cytoplasmic translocation of vitellogenin to forming yolk bodies concomitant with proteolytic cleavage of vitellogenin into the polypeptide subunits of the yolk proteins, lipovitellin and phosvitin [17]. Some complexity has been introduced into this general

A-gold. Arrowheads indicate immunoreactivity within Golgi saccules. $\times 23,520$.

FIG. 12. The relationship of cortical alveoli (“yolk vesicles”) in oocytes and cortical alveoli in eggs. These sections were embedded in Histo-resin prior to treatment with antibodies. a: Light micrograph of a section through the ovary of *F. heteroclitus* that was treated with anti-cortical alveolus antibody (see above) followed by treatment with horseradish peroxidase-labeled secondary antibody. Peroxidase reaction product is visible only within the cortical alveoli (“yolk vesicles” (arrows)) of various size oocytes and is not present in smaller (pre-cortical alveolus-stage) oocytes (O). $\times 80$. b: This section through an egg of *F. heteroclitus* demonstrates immunoreactivity only in the cortical alveoli (arrows). V, vitelline envelope; Y, yolk mass. $\times 85$.

scheme in recent years with the realization that vitellogenin in some species may be encoded by multiple, closely related genes [63, 64], so that multiple, closely related yolk polypeptides may accumulate in the oocyte [65, 66].

The biochemical and cytological details of vitellogenesis in fish have not been as forthcoming. But, since this subject was last reviewed [9-11, 16], previously unemphasized or new observations have been made. Vitellogenin has been identified in the plasma or serum from females or estrogen-treated males of a variety of teleosts (Fig. 13, [44, 67-81]) and a partial characterization has been

made of vitellogenin in trout [82]. Extracellular tracers have been used to follow protein transport from the perfollicular capillaries to the oocyte surface via patent intercellular channels of the follicular epithelium (Fig. 8, [44, 83-85]) and vitellogenin transport to forming yolk bodies has been documented [44, 83]. Figure 14 is an electron micrograph of the surface of a vitellogenic oocyte and reveals much pinocytotic activity, which is typical of the oocyte during this stage of growth. One study that has attempted to demonstrate selective uptake of vitellogenin by trout oocytes *in vitro* [86] is unconvincing when the results are recalculated on a molar basis [17] and the only demonstration of selective uptake in teleosts is a recent report by Tyler *et al.* [87] in which they observe that *in vitro* rates of vitellogenin uptake in the rainbow trout, *S. gairdneri*, are comparable to those observed for the amphibian, *Xenopus laevis*. Using [³²P]vitellogenin, Wallace and Begovac [88] have shown that vitellogenin gives rise to at least the phosvitin polypeptides in *F. heteroclitus*, and more recently Tyler *et al.* [81] demonstrated in *S. gairdneri* that both lipovitellin and phosvitin are vitellogenin-derived. However, several studies also exist that claim vitellogenin is not processed into smaller yolk polypeptides after being taken up by the teleost oocyte [89, 90 (but see 91), 92]. Several descriptive studies in teleosts have documented that multivesicular (or lysosome-like) bodies are prominent within oocytes when vitel-

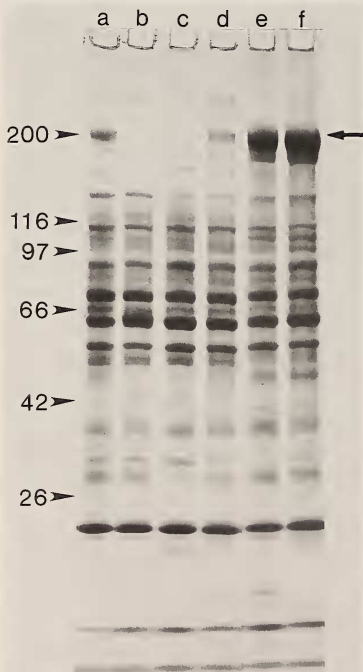


FIG. 13. Estrogen induction of vitellogenin in male *F. heteroclitus*. Plasma from female (lane a), male (lane b) and estrogen-treated males (lanes c-f) were analyzed by SDS-polyacrylamide gel electrophoresis on linear gradient gels (6.93-20.44% acrylamide). Estrogen-treated males, maintained at 28-30°C, were given a single injection of 20 µg 17β-estradiol dissolved in 20 µl peanut oil 6 hr (lane c), 12 hr (lane d), 24 hr (lane e) and 72 hr (lane f) prior to bleeding. All fish were injected with 0.1 ml aprotinin solution (17 trypsin inhibitor units/ml) 15 min prior to bleeding to suppress proteolysis during blood collection. The 200-kDa polypeptide, vitellogenin (arrow), is present in female (lane a) but not male (lane b) plasma and is apparent in males 12 hr after estrogen administration (lane d). Molecular mass values are indicated in kDa on the left. Each lane contains 1 µl plasma.

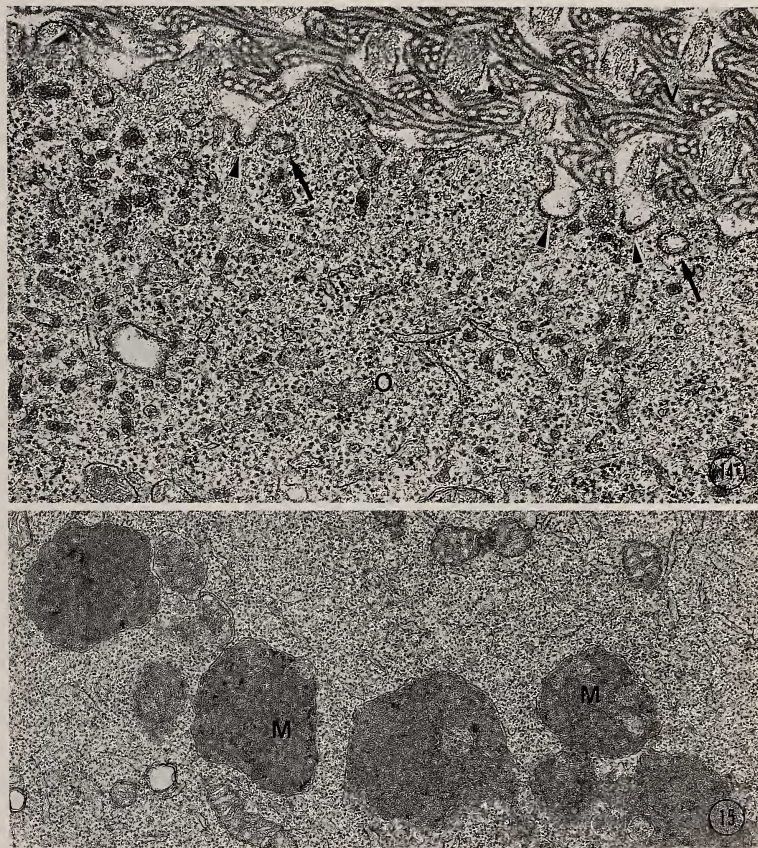


FIG. 14. Electron micrograph of the surface of an oocyte (O) from an early vitellogenic follicle from the sheephead minnow, *Cyprinodon variegatus*, showing abundant endocytotic activity. Coated pits (arrowheads), coated vesicles (arrows) and smooth-surfaced tubules are numerous at the oocyte surface. V, vitelline envelope. $\times 35,280$. From Selman and Wallace [83].

FIG. 15. Multivesicular-like bodies (M) in the cytoplasm of a cortical alveolus-stage oocyte from *C. variegatus* that was injected with the electron dense tracer iron dextran, 20 hr prior to sacrifice. The exogenous tracer was removed from the maternal circulation via endocytosis at the oocyte surface and subsequently translocated to multivesicular-like bodies within the oocyte. The dense particulate matter within these bodies is iron dextran. $\times 20,000$.

logenesis begins and that these structures disappear as yolk bodies accumulate within growing oocytes (Fig. 15, [7, 83, 93]). More detailed studies in *X. laevis* have demonstrated that multivesicular bodies do indeed play a key role in vitellogenin processing within oocytes [94, 95]. Clearly, the major areas requiring further inves-

tigation in teleosts are the selective uptake, translocation, and processing of vitellogenin. The development of appropriate culture procedures and methods of removing the surface epithelium from ovarian follicles will be a prerequisite for such studies.

Yolk proteins of several teleosts have been

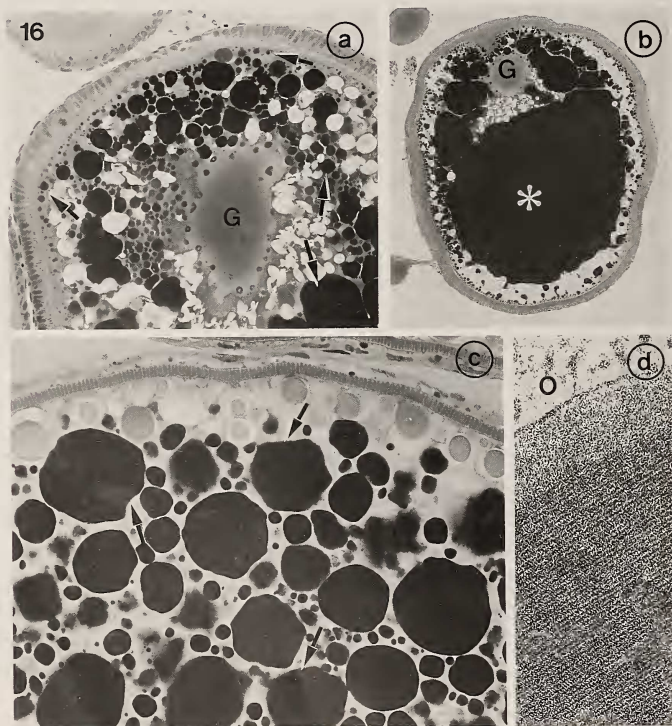


FIG. 16. Yolk accumulation within yolk spheres (panel a) that coalesce to form a continuous mass of fluid yolk (panel b) and within platelets (panel c) that have a crystalline core (panel d). Panels a, b: These sections of mid- and late vitellogenic follicles, respectively, from *C. variegatus* show fluid yolk bodies (arrows) in various states of fusion. Note the central mass of fluid yolk (*) occupying much of the ooplasm in the oocyte in panel b. G, germinal vesicle. Panel a: $\times 215$; Panel b: $\times 62$. Panel c: This section through a mid-vitellogenic follicle from the zebrafish, *Brachydanio rerio*, reveals numerous yolk platelets of variable size (arrows). $\times 572$. Panel d: This electron micrograph shows the crystalline core of a yolk platelet from the mullet, *Mugil cephalus*. O, ooplasm. $\times 108,000$.

isolated and/or partially characterized in recent years, primarily by gel electrophoretic and chromatographic procedures [68, 78, 80, 88, 96-98]. A detailed comparative study by Lange and colleagues [99, 100] has documented that the yolk proteins of two freshwater teleosts, as well as several other actinopterygians [101, 102] are present in platelets (Fig. 16c, d) comprised of an orthorhombic crystalline structure, as is found in all other anamniotes except cyclostomes. A unique feature of many teleosts is that yolk proteins accumulate in fluid-filled yolk spheres or "yolk globules" (Fig. 16a), rather than crystalline platelets [103]. These yolk spheres may either maintain their integrity throughout oocyte growth [104] or fuse centripetally to form a continuous mass of fluid yolk (Fig. 16b, [9]), a process that confers on many teleost eggs their characteristic transparency. This fusion can occur relatively soon after the initial formation of yolk spheres, as in sticklebacks and pipefish (our unpublished observations), during the later stages of vitellogenesis as in the sheephead minnow, *Cyprinodon variegatus* [9], or during maturation as in those teleosts that have pelagic eggs [105-107]. As a consequence of yolk fusion, the ooplasm is displaced into a peripheral rim surrounding the yolk mass (Fig. 16b).

MATURATION

The following events characterize the resumption of meiosis that occurs during oocyte maturation: a) the germinal vesicle migrates towards the periphery of the oocyte and the nuclear envelope dissociates; b) the chromosomes condense and proceed to first meiotic metaphase, followed by the elimination of the first polar body; and c) the remaining chromosomes then proceed to the second meiotic metaphase, where they arrest once again [108]. In teleosts, as in other vertebrates, once this second arrest occurs (and not before), the oocyte has become "mature" and fertilizable, and now can correctly be called an egg. Ovulation of the oocyte usually occurs towards the end of the maturation process. In recent years, studies on oocyte maturation, especially in amphibians, have given considerable attention to the sequence of

cytoplasmic events responsible for the reinitiation of meiosis and to the waxing and waning of cytoplasmic factors responsible for nuclear and chromosomal behavior during this stage [109, 110]. Aside from hormonal signals associated with oocyte maturation [13, 19], virtually nothing is known about similar processes in teleosts.

In most animals, oocytes reach their final size during vitellogenesis and undergo maturation and ovulation, after an appropriate hormonal stimulus. This is the situation for most freshwater teleosts. Oocyte maturation in teleosts is usually quite rapid and accomplished within 24 hr (depending on species, temperature, etc.). In some, particularly marine teleosts, there is also an enormous volume increase due to rapid water uptake, which occurs concomitant with oocyte maturation [9]. Measurements made by Fulton [105] on two dozen different marine teleosts indicated increases in oocyte volume during maturation ranging from three- to four-fold. More recently, several authors have noticed that ovarian hydration occurs either after injection of maturation-inducing hormones to gravid fish ([111], see also references in [9]) or during the normal course of oocyte maturation [112-114]. Correlated with hydration were increases in total K^+ [111, 113] and in protein dephosphorylation [114].

Maturation accompanied by normal hydration has also been achieved *in vitro* for oocytes isolated from a variety of fish (Fig. 17, [115, 116]). Observations initially made on *F. heteroclitus* indicated that proteolysis of yolk proteins also occurs during maturation *in vivo* or *in vitro* (Fig. 18, [88, 97]). Greeley *et al.* [116] subsequently surveyed a variety of teleosts whose oocytes increased in volume from 0% to 643% and found that the extent of yolk proteolysis correlated well with the extent of oocyte hydration. More recently, we demonstrated that oocyte maturation could be dissociated from hydration but not proteolysis by incubating steroid-treated follicles of *F. heteroclitus* in paraffin oil [117]. Thus, hydration is unnecessary for maturation and proteolysis, but the dependence of hydration on maturation or proteolysis is as yet uncertain.

In summary, for some teleosts oocyte hydration during maturation accounts for up to 86% of the

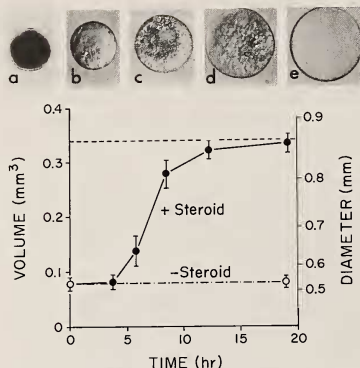


Fig. 17. Size change which occurs in the oocytes of the cunner, *Tautoglabrus adspersus*, during maturation. The largest follicles present in the ovary were incubated at 20°C in the presence (solid line) or absence (stippled line) of maturation-inducing steroid. Each point represents the average size of 10 follicles $\pm$ SD. Dashed horizontal line at 0.87 mm indicates the average size of 10 eggs collected from the ovarian lumen. Photomicrographs at top depict the appearance of individual follicles with diameters of (a) 0.50 mm, (b) 0.55 mm, (c) 0.67 mm (GV apparent near center), (d) 0.82 mm (GV approaching animal pole) and (e) 0.85 mm (GV no longer apparent and ooplasm has clarified). Adapted from Wallace and Selman [9].

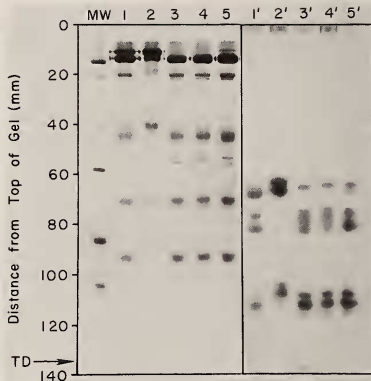
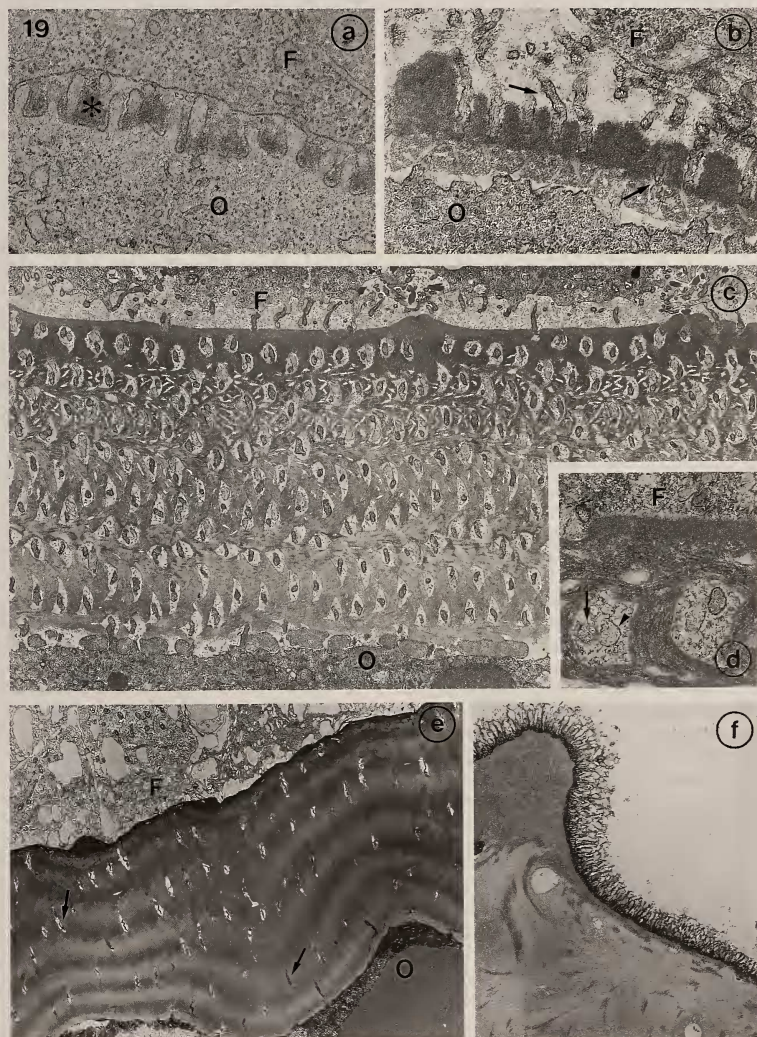


Fig. 18. SDS-polyacrylamide electrophoresis on 12% gels of the major proteins present in extracts of ovary (lanes 1 and 1'), prematurational (post-vitellogenic) follicles (lanes 2 and 2'), postmaturation follicles matured *in vivo* (lanes 3 and 3') or *in vitro* (lanes 4 and 4') and ovulated eggs (lanes 5 and 5'). Lanes 1-5 represent samples containing approximately 50 μ g protein and are stained with Coomassie Blue, as is the first lane that indicates molecular mass standards (92.5, 66.2, 45.0, 31.0, 21.0 and 14.4 kDa from top to bottom respectively). Lanes 1-5 represent samples containing approximately 100 μ g protein electrophoresed under identical conditions but stained with Stains-all to demonstrate acidic phosphoproteins. From Wallace and Begovac [88].

final egg size. As a consequence, such eggs are rendered buoyant in sea water [105] and thus become widely dispersed. It appears that the accumulation of K^+ and/or protein dephosphory-

lation may contribute the osmotic effectors involved in this remarkable phenomenon. The occurrence of yolk protein proteolysis during oocyte maturation helps to explain the presence of

Fig. 19. Vitelline envelope formation in *F. heteroclitus*. Panel a: Electron-dense envelope material (*) begins to accumulate between microvilli that extend from the surface of the oocyte (O) towards the overlying follicle cells (F) within a primary growth stage follicle. $\times 42,000$. Panel b: Envelope material continues to accrue and become bilaminar in appearance as oocyte growth continues. Microvillar processes (arrows) extending both from the oocyte (O) and follicle cells (F) can be seen penetrating the developing envelope. $\times 29,700$. Panel c: The vitelline envelope in this late vitellogenic follicle has greatly increased in size and complexity and has become multilaminar. Microvillar processes from both the oocyte (O) and follicle cells (F) are apparent within patent pore canals that traverse the developing vitelline envelope. $\times 5,280$. Panel d: Higher power electron micrograph demonstrating pore canals that contain both oocyte (arrow) and follicle cell (arrowhead) processes. F indicates cytoplasm of a follicle cell. $\times 22,400$. Panel e: The vitelline envelope in this late maturational follicle has undergone compaction and has become more homogenous in appearance. The pore canals (arrows) become narrower as the microvillar process from both the oocyte (O) and follicle cells (F) begin to retract. $\times 2,950$. Panel f: The outer region of the vitelline envelope (chorion) from a mature egg displays a filamentous coat. $\times 10,500$.



unusual yolk protein polypeptides present in the eggs of many, particularly marine teleosts [17]. However, the molecular and physiological mechanisms responsible for oocyte hydration during maturation remain to be elucidated.

OTHER CELLULAR ASPECTS OF OOCYTE GROWTH

Vitelline envelope formation

The vitelline envelope begins to form during the perinucleolus stage of oocyte growth and continues to increase in size and differentiate throughout the remainder of the oocyte growth stages (Fig. 19). As envelope material continues to accrue extracellularly, the microvillar processes extending from both the oocyte and follicle cells increase in length and reside within pore canals that penetrate the enlarging envelope. Gap junctions have been identified between microvillar processes of both cell types on the distal side of the vitelline envelope [8, 118–120]. As the follicle grows, the vitelline envelope becomes highly ordered and architecturally complex; particularly good descriptions of this process are provided by Azevedo [2], Wourms [3], Ulrich [57], Anderson [121], Busson-Mabillot [122], Riehl [123], and Stehr and Hawkes [124]. Most studies on the vitelline envelope are of a morphological nature, but several recent reports have described the biochemical composition of these intricate structures [125–127]. One important unanswered question regarding the vitelline envelope in teleosts is which cells, the oocyte and/or the follicle cells, are synthesizing its components? Immunochemical studies in the medaka, *Oryzias latipes*, indicate that the liver contributes to its formation, at least in this species [128].

Lipid formation

In most teleosts, lipid bodies first appear in cortical alveolus-stage oocytes, where they reside in the perinuclear ooplasm (Fig. 20). Lipid continues to amass throughout most of oocyte growth and more variation occurs in this process among fish species than for previously mentioned events. Examples can be cited ranging from fish eggs

devoid of lipid droplets, such as in the cunner, *Tautoglabrus adspersus* [9, 129], to gravid ovaries of the gourami, *Trichogaster cosbyi*, in which 37% of the weight is wax ester [130, 131]. The latter fish is typical of the bubble nest builders, which spawn buoyant eggs in fresh water [132]. It should be noted that lipid is frequently hard to detect in histological preparations since it is dissolved during routine histological processing of tissue. Thus, unless fixatives are used that specifically retain lipids (e.g., osmium tetroxide (Fig. 9)), it is difficult to distinguish lipid droplets from vacuolar appearing cortical alveoli (Fig. 20).



FIG. 20. Light micrograph of a section through a cortical alveolus-stage follicle from *F. heteroclitus*. This tissue was fixed in a solution that did not contain osmium tetroxide and thus it is difficult to distinguish the lipid droplets (arrowheads) from cortical alveoli (*) because the contents of both are leached out during tissue preparation (compare with Fig. 9). Although much lipid is located in a perinuclear position, lipid bodies that are more randomly arranged throughout the ooplasm are difficult to distinguish. This section was stained with toluidine blue. $\times 180$.

Determination of egg size

The final egg size can vary considerably among teleosts, the extremes for North American waters ranging from less than 0.3-mm diameter for the sea perch, *Cymatogaster aggregatus* [133], up to 30-mm diameter for the marine catfish, *Bagre marinus*

[134]. The most effective way to achieve disparate egg size would be to modify yolk accumulation or hydration. The published pictures of *C. aggregatus* eggs seem to indicate that oocytes do not advance beyond the cortical alveolus-stage, i.e., neither vitellogenesis or terminal hydration during maturation take place [135, 136]. If so, it would be interesting to explore whether these animals have lost the capacity to make vitellogenin or whether oocyte sequestration is deficient. The available evidence for *B. marinus* is inconclusive, but it would appear that its eggs are fully yolked, i.e., they are opaque and do not display the transparent quality of eggs that have undergone enlargement due to hydration.

A NEW PERSPECTIVE OF OOCYTE STAGES

Many staging systems of oocyte development focus on a specific event (or events) that characterizes a certain size range of oocytes with the implication being that only within this size class of oocytes does the specific event (or events) occur. As we have learned more about the cellular and biochemical processes that are involved in oocyte growth and have developed procedures to quantify such events, it has become quite apparent that the synthesis or accumulation of various materials is not necessarily stage-specific, as is implied by most oocyte staging systems. Some examples are:

- 1) For years it was widely held that lampbrush chromosomes were engaged in the synthesis of maternal mRNA only during the later part of primary oocyte growth, when such chromosomes are readily perceived and by the end of which the full complement of maternal mRNA has accumulated [137]. Nevertheless, in *X. laevis* lampbrush chromosomes have been demonstrated in late vitellogenic oocytes [138] and poly(A)-containing RNA continues to be synthesized in the absence of long-term accumulation throughout oocyte growth, suggesting that the level of maternal mRNA present at various stages of oocyte growth is the result of synthesis combined, in later stages, with turnover [139].

- 2) Vitelline envelope formation begins during the primary growth stage and appears to continue throughout the rest of oocyte growth (Fig. 19).

- 3) [^3H]Glucose has been found to label exclusively the glycoconjugate present in *F. heteroclitus* cortical alveoli during short-term pulse experiments, as demonstrated by autoradiographic (Fig. 10) and electrophoretic procedures [42]. When follicles of various sizes were incubated for short periods with [^3H]glucose, incorporation was greatest in cortical alveolus-stage oocytes for results expressed on a per volume basis [42], as expected from microscopic impressions. However, when the results were expressed on a per follicle basis, absolute rates of incorporation were found to be greater in vitellogenic and maturation-stage oocytes (Fig. 21). The massive accumulation of yolk protein and water during these later stages thus subjectively appeared to relegate the synthesis of cortical alveolus material to a trivial or nonexistent process when perceived cytologically.

- 4) The sequestration of vitellogenin has been found to occur not only during vitellogenesis *per se*, but also during at least the first part of maturation in both amphibians [140] and fish (Fig. 21, [44]). Pinocytotic activity is apparent at the oocyte surface throughout vitellogenesis and early maturation and the protein content of developing oocytes continues to increase concomitant with maturation [97].

Because of these and other observations, we would like to advance the notion that physiological events do not sequentially replace one another during oocyte growth, but rather that these events are sequentially initiated and, once initiated, remain active throughout oocyte development (Fig. 21). Oocyte growth and all the processes previously initiated may accelerate or slow down due to a regimen of hormonal clues, or even turn off during atresia, but overall oocyte development is considered to comprise a cascade of cellular processes that occur during meiotic arrest. Oocyte maturation appears to represent a true change in oocyte physiology since after germinal vesicle breakdown, surface projections from the oocyte retract and endocytosis no longer continues [97, 140] as the oocyte finally ovulates from its follicular investments and changes into an egg.

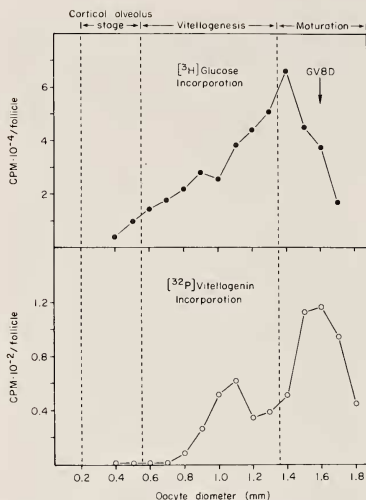


FIG. 21. Cortical alveoli formation and vitellogenesis. Top panel: Incorporation of $[^3\text{H}]$ glucose into TCA-precipitable material in different sized follicles from *F. heteroclitus* that were incubated for 12 hr in fish saline containing $[^3\text{H}]$ glucose, followed by a 12 hr incubation in saline solution alone. The data are expressed as cpm/follicle and indicate that a glycoconjugate component of cortical alveoli is made throughout vitellogenesis and early maturation. Adapted from Selman *et al.* [42]. Lower panel: Distribution of *F. heteroclitus* $[^{32}\text{P}]$ vitellogenin within different sized follicles 20 hr after injection into a single female. Sequestration of vitellogenin occurs during vitellogenesis and continues through early maturation. Adapted from Selman and Wallace [44].

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